

HAEMATOLOGICAL AND BIOCHEMICAL BLOOD PROFILE OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) CULTURED IN PONDS OF DIFFERENT WATER DEPTH AND FED SINKING VERSUS FLOATING DIET

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Abstract: This study contributes data on haematological and biochemical parameters of African catfish, *Clarias gariepinus*. It employed a 3×2 factorial design with three ponds of different water depth (0.5, 1 and 1.5 m) and two types of feed (floating and sinking). Twelve earthen ponds (1 m x 2 m) were stocked with 16 fingerlings catfish each (mean weight ~100g) and their blood parameters were monitored over 12 weeks. Differences in hematological parameters related to water depth were mostly significant, and better results were recorded in fish reared in shallower water ponds. Feed type showed improved hematological parameters with using of sinking diet. Most biochemical parameters showed significant differences in pond waters depth and feed type with better results coincided with rearing fish in shallower water depth and with sinking feed. Conclusively, culturing Catfish in shallow ponds (0.5 m) and use of sinking feed improve physiological response and health condition.

Key words: pond water depth, floating and sinking fish feed, African catfish (*Clarias gariepinus*), physiological response

Introduction

Intensive aquaculture production of African catfish (*Clarias gariepinus*) has increased in the last few decades. Although intensive production delivers the maximum benefits from cultured area but it subject the fish to many stressors as high stocking density, handling, transportation, bad water quality, sorting and

grading (*Thanikachalam et al., 2010*). In order to overcome these stressors and improve fish health and welfare, several attempts have been done. For example, improve husbandry system used for fish rearing, use optimum nutrient level, improve water quality and the using of immunostimulants and growth promoters (*Gabriel et al., 2019*).

Haematological and biochemical studies of cultured fish are important in order to monitor the health of fish during cultivation. Such studies are particularly useful in assessing a fish's physiological and physiopathological status since morphological and biometric parameters alone do not always give a complete picture (*Adakole, 2012; Tavares-Dias and Moraes, 2007*). However, in order for data on haematological and biochemical parameters to be meaningful, there have to be reliable reference ranges for comparison. For a number of fish species, there is still a scarcity of studies establishing normal blood values and reference ranges. Normal values exist for only a handful of hematological parameters and the established values tend to have a wide range due to a lack of standardization between methods (*Fazio et al., 2019*). This study contributes important data on the normal blood parameters of African catfish (*Clarias gariepinus*) for which little data exists. The data from this study are part of a study on the effect of water depth and sinking versus floating feed on the growth of catfish (*Abdel-Hay et al., 2019*).

Materials and Methods

Ethical approval

Ethical approval for this study was obtained from the Committee of Aquatic Animal Care and Use in Research, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt.

Experimental design

A 3×2 factorial treatment design was used to evaluate the effect of three pond water depth (0.5, 1 and 1.5 m) and two types of feed (floating and sinking) on the growth performance of African catfish. There were two replicates per treatment and the study was carried out for 12 consecutive weeks. These water levels were chosen to reflect the traditional water depth used throughout Africa of approximately 1 m (*Hecht et al., 1996*).

Animal husbandry system

The experiment was conducted in 12 equal-sized earthen ponds (1 m x 2 m) with different water depth (0.5, 1 and 1.5 m). The animals were exposed to the following treatments; one receiving floating feed and the other sinking feed at three water depths (0.5, 1 and 1.5 m), with two replicates each. Water exchange was carried out every two days at a rate of 5 – 10%. Each pond was randomly stocked

with 16 animals at an average weight of 100.15 ± 3.480 g. The animals were left to acclimatize for one week prior to the start of the experiment. All animals were fed a quantity of approximately 3% of body mass each morning for 12 weeks and were weighed every three weeks at which point feeding rate was adjusted accordingly. Ethical approval for this study was obtained from the Committee of Aquatic Animal Care and Use in Research, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt (approval number: IAACUC-KSU-2-2018).

Experimental feeds

A commercial floating and sinking feed (Table 1) were purchased from a local feed factory (Al-Ekhwa[®] feed factory, Kafrelsheikh, Egypt). The content of both feeds was identical, and they differed only in form (floating versus sinking).

Table 1. Composition and chemical analysis of the experimental diets on dry matter basis

	Ingredients composition (%)	
Fish meal (72%CP)	10	10
Soybean meal (45%CP)	40	40
Yellow corn	24	24
Wheat bran	10	10
Rice bran	10	10
Corn oil	3	3
Di calcium phosphate	1	1
Vitamin and mineral mixture	2	2
Total	100	100
	Chemical analysis (%)	
Dry matter (DM %)	93.00	92.15
Crude protein (CP %)	30.85	30.84
Ether extract (EE %)	7.94	7.91
Crude fiber (CF %)	4.95	5.01
Ash %	8.66	8.29
Nitrogen free extract (NFE %)	48.00	47.85
	Calculated energy value	
Gross energy (kcal/kg)	4496.36	4492.48
Digestible energy (kcal/kg)	3372.27	3394.36
Metabolizable energy (Kcal/kg)	365.63	362.09

Haematological and biochemical parameters

Blood sampling and serum separation

Blood samples were taken from the caudal vein of 16 animals in each treatment (eight fish per replicate) using a sterile syringe. Each sample taken was

split in two: the first part was transferred into a sterile 2 ml test tube with added KEDTA for future analysis in a haematological assay and the second part was stored in a 2 ml Eppendorf tube which would be later used for serum separation. Blood was left to coagulate at 4°C for 60 minutes. After that, tubes were centrifuged at 3000 rpm for 10 minutes in order to separate serum which was then transferred into clean Eppendorf tubes and stored at -40°C until the time of analysis.

Haematological parameters

The following blood parameters were measured: red blood cell count (RBC), haemoglobin concentration (HB), packed cell volume (haematocrit), mean corpuscular volume (MCV), mean corpuscular haemoglobin count (MCH), mean corpuscular haemoglobin concentration (MCHC) and white blood cell count (WBC) using an automatic blood cell counter (Exigo-Vet., Boule Medical AB Inc., Stockholm, Sweden). For estimation of the differential leucocytes count, two thin smears were prepared from each blood sample on clean microscope slides and were left to air-dry before being stained with a modified Wright's stain and covered. A total of 100 cells were counted under a $\times 100$ oil immersion lens and the percentage of heterophils, lymphocytes, and monocyte was estimated following the method outlined by *Anderson and Siwicki (1995)*.

Biochemical parameters

Serum total protein (TP) was determined calorimetrically using commercial kits (TP0100, Sigma-Aldrich, USA). Serum albumin was measured using the bromocresol green binding method (*Doumas et al., 1971*). Serum globulin was calculated by subtracting albumin values from total protein. Albumin/globulin (A/G) ratio was calculated by dividing albumin values by globulin values. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine were assessed following the method outlined in *Palti et al. (1999)*.

Statistical analysis

Data were tested for distribution normality, linearity and homogeneity of variance. Log-transformation of the raw data was used for some measured parameters because of the large range across the data. Data were analysed and visualized in GraphPad Prism 6 and all results were reported as means with SEM. A two-way ANOVA was used for comparison of the main effects of pond water level and feed type. The interaction of the two factors was tested using Tukey's multiple comparison test as a *post hoc* test where appropriate. The level of significance was set at $p \leq 0.05$.

Results and Discussion

Haematological parameters

Differences in blood cell formation and function are considered as good indicators of nutritional status, stress response, health condition and welfare of fish (*Buentello et al., 2007*). Most blood parameters of the animals in this study differed significantly between the different treatments except for red blood cell count (RBCs), mean corpuscular volume, and mean corpuscular haemoglobin and concentration (see Table 2). The highest levels were recorded in the shallowest water for the following parameters: haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), and lymphocytes whilst the lowest values were recorded for the following parameters: white blood cells (WBC) and heterophils. Differences in blood parameters between animals fed different feed types were not significant except for WBCs, heterophil percentage, lymphocytes and monocytes. Higher values of haematological parameters were recorded in the animals fed sinking pellets for Hb, PCV, MCV, MCH, MCHC, heterophils, and monocytes while lower values were recorded for RBC, WBC, and lymphocytes. The interaction between pond water levels and feed type was not significant for most blood parameters except WBC, lymphocytes, heterophils, and monocytes.

The results from this study are similar to those reported by *Owolabi (2011)*, *Al-Dohail et al. (2009)* and *Acharya and Mohanty (2014)*. All haematological parameters in the present study are within the normal reference range of catfishes (*Lim et al., 2000; Owolabi, 2011*). This indicates that both the pond water levels and feed types used in this study meet the basic needs of the African catfish. An erythrogram (RBC, Hb, PCV, MCV, MCH, MCHC) is commonly used to detect conditions such as anaemia and other basic health issues in animals (*Owolabi, 2011*) while, the leukogram (WBC, lymphocytes, heterophils, and monocytes) is used to get a picture of the status of an animal's immune system (*Fagbenro et al., 2000*). The increase in the values of the erythrogram in the animals reared in the shallowest ponds in this study (0.5 m) may be related to an increase in fish activity (*Acharya and Mohanty, 2014*) and improved health condition (*Buentello et al., 2007*). The results from this section of the study would imply that African catfish attain optimal physiological status in 0.5m deep ponds; however, larger sample sizes and more detailed studies would be needed to explore this further.

Table 2. Effect of pond water level and feed type on the hematological parameters of African catfish, *Clarias gariepinus*

	Hb (g/l)	RBC ($10^6/L$)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/l)	WBC ($10^3/L$)	Heterophils (%)	Lymphocytes (%)	Monocytes (%)
Water level										
0.5 m	11.93 ^a	3.533	36.63 ^a	105.7	35.53	33.52	34.68 ^b	3.333 ^b	90.83 ^a	5.917 ^b
1.0 m	10.88 ^b	3.650	33.63 ^b	93.59	30.55	32.32	41.03 ^a	4.625 ^{ab}	86.42 ^b	8.958 ^a
1.5 m	10.38 ^b	3.258	31.58 ^b	97.41	31.98	32.86	35.13 ^b	5.417 ^a	89.42 ^b	5.167 ^b
Feed type										
Floating	10.92	3.489	33.56	97.62	31.98	32.56	37.17	3.556	90.78	5.778
Sinking	11.02	3.472	34.34	100.2	33.40	33.24	36.73	5.361	87.00	7.583
SEM	0.384	0.203	1.159	6.460	2.456	0.512	1.236	0.543	1.516	0.429
P-value										
Water level	0.002*	0.169	0.001*	0.190	0.142	0.091	0.001*	0.004*	0.027*	0.001*
Feed type	0.194	0.921	0.419	0.637	0.487	0.120	0.669	0.001*	0.007*	0.001*
Interaction	0.386	0.396	0.780	0.377	0.497	0.113	0.006*	0.024*	0.048*	0.001*

Means within a column and effect that lack common superscripts differ significantly (Tukey's multiple comparison test, $p \leq 0.05$). Asterisks indicate significant differences between groups (two-way ANOVA* $p \leq 0.05$). SEM= standard error of the mean.

Hb=hemoglobin; RBCs=red blood cells; PCV=packed cell volume; MCV=mean corpuscular volume; MCH=mean corpuscular hemoglobin; MCHC=mean corpuscular hemoglobin concentration; WBCs=white blood cells

Biochemical parameters

All serum biochemical parameters in the animals in this study differed significantly between the different water level treatments except for total protein (TP) and creatinine (see Table 3). The highest value of serum biochemical parameters were recorded in the fish kept in shallowest ponds for the following parameters: TP and albumin while, globulin, ALT, AST, cholesterol, triglycerides and glucose recorded the highest values in deeper ponds. Differences in serum biochemical parameters between animals fed floating and sinking pellets were significant for all parameters except for TP, albumin, cholesterol and creatinine. Higher serum biochemical parameters were recorded in the animals fed floating pellets for albumin, albumin-to-globulin (A/G) ratio, ALT, AST, triglycerides and creatinine while the lower values were recorded in the following parameters: TP, globulin, cholesterol and glucose. The interaction effect between pond water level and feed type was significant for all biochemical parameters except for TP, globulin and creatinine.

These results are similar to those reported by *Al-Dohail et al. (2009)*, *Owolabi (2011)* and *Acharya and Mohanty (2014)*. All biochemical parameters in this study are within the normal reference range of catfish (*Owolabi, 2011*). This indicates that both the pond water levels and feed types used in this study meet the

basic needs of the African catfish. The fish reared in the shallowest ponds and fed sinking pellets had the highest TP and globulin levels. These biochemical parameters are all known to play an important role in fish nutrition, health and immunity (Swain, 2007). In contrast, the other biochemical parameters in fish reared in the shallowest ponds and fed sinking pellets were higher than the other groups but still within the normal range.

Table 3. Effect of pond water level and feed type on the serum biochemical parameters of African catfish, *Clarias gariepinus*

	TP (g/dl)	Albumin (g/l)	Globulin (g/dl)	A/G ratio	ALT (U/L)	AST (U/L)	Cholesterol (mmol/l)	Triglycerides (mmol/l)	Glucose (mmol/l)	Creatinine (mmol/l)
Water level										
0.5 m	5.360	2.169 ^a	3.191 ^a	0.695 ^{ab}	61.90 ^b	446.6 ^c	405.6 ^b	69.80 ^b	36.40 ^b	0.377
1.0 m	5.080	2.073 ^{ab}	3.007 ^b	0.705 ^a	66.90 ^b	585.9 ^b	440.2 ^b	59.60 ^c	45.00 ^b	0.441
1.5 m	5.240	1.980 ^b	3.260 ^a	0.637 ^b	74.50 ^a	661.0 ^a	575.0 ^a	88.60 ^a	58.20 ^a	0.425
Feed type										
Floating	5.260	2.100	3.067	0.706	82.13	819.6	462.0	79.73	31.87	0.441
Sinking	5.460	2.048	3.239	0.653	53.40	309.4	485.2	65.60	61.20	0.388
SEM	0.133	0.047	0.051	0.024	2.624	27.97	24.60	4.043	3.555	0.037
P-value										
Water level	0.128	0.002*	0.001*	0.018*	0.003*	0.001*	0.001*	0.001*	0.001*	0.225
Feed type	0.279	0.189	0.001*	0.012*	0.001*	0.001*	0.259	0.003*	0.001*	0.097
Interaction	0.298	0.006*	0.277	0.004*	0.001*	0.025*	0.012*	0.039*	0.001*	0.311

Means within a column and effect that lack common superscripts differ significantly (Tukey's multiple comparison test, $P \leq 0.05$). Asterisks indicate significant differences between groups (two-way ANOVA * $p \leq 0.05$). SEM= standard error of the mean.

TP= total protein; A/G ratio= albumin-to-globulin ratio; GPT= glutamic pyruvic transaminase; GOT= glutamic oxaloacetic transaminase.

Conclusion

Rearing African Catfish in shallow ponds water depth (0.5 m) and the use of sinking feed significantly improve its physiological response, health condition and welfare.

Hematološki i biohemijski profil krvi afričkog soma (*Clarias gariepinus*) uzgajanog u ribnjacima različite dubine vode i hranjenog tonućim odnosno plutajućim obrokom

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Rezime

Ova studija daje podatke o hematološkim i biohemijskim parametrima krvi afričkog soma, *Clarias gariepinus*. Korišćen je faktorski dizajn 3×2 sa tri ribnjaka različite dubine vode (0,5, 1 i 1,5 m) i dve vrste hrane (plutajuća i tonuća). Dvanaest zemljanih ribnjaka (1 m x 2 m) bilo je opskrbljeno sa po 16 somova (prosečna težina ~ 100 g) i praćeni su njihovi parametri krvi tokom 12 nedelja. Razlike u hematološkim parametrima u vezi sa dubinom vode bile su uglavnom značajne, a bolji rezultati zabeleženi su kod riba uzgajanih u plićim vodenim ribnjacima. Tip hrane je pokazao poboljšane hematološke parametre uz upotrebu tonućeg obroka. Većina biohemijskih parametara pokazala je značajne razlike sa stanovišta i dubini vode u ribnjaku i tipu hrane, sa boljim rezultatima koji su se podudarali sa uzgojem ribe u manjoj dubini vode i sa tonućom hranom. Zaključno, uzgoj soma u plitkim ribnjacima (0,5 m) i upotreba tonuće hrane poboljšavaju fiziološki odgovor i zdravstveno stanje.

Ključne reči: dubina vode u ribnjaku, plutajuća i i hrana koja tone, afrički som (*Clarias gariepinus*), fiziološki odgovor

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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